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Intrafamilial Correlation of Clinical Manifestations in Neurofibromatosis 2 (NF2)

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Measuring correlation in clinical traits among relatives is important to our understanding of the causes of variable expressivity in Mendelian diseases. Random effects models are widely used to estimate intrafamilial correlations, but such models have limitations. We incorporated survival techniques into a random effects model so that it can be used to estimate intrafamilial correlations in continuous variables with right censoring, such as age at onset. We also describe a negative-binomial gamma mixture model to determine intrafamilial correlations of discrete (e.g., count) data. We demonstrate the utility of these methods by analyzing intrafamilial correlations among patients with neurofibromatosis 2 (NF2), an autosomal-dominant disease caused by mutations of the *NF2* tumor-suppressor gene. We estimated intrafamilial correlations in age at first symptom

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of NF2, age at onset of hearing loss, and number of intracranial meningiomas in 390 NF2 nonprobands from 153 unrelated families. A significant intrafamilial correlation was observed for each of the three features: age at onset (0.35; 95% confidence interval (CI) 0.23–0.47), age at onset of hearing loss (0.51; 95% CI, 0.35–0.64), and number of meninginomas (0.29; 95% CI, 0.15–0.43). Significant correlations were also observed for age at first symptom within NF2 families with truncating mutations (0.41; 95% CI, 0.06–0.68) or splice-site mutations (0.29; 95% CI, 0.03–0.51), for age at onset of hearing loss within families with missense mutations (0.67; 95% CI, 0.18–0.89), and for number of meningiomas within families with splice-site mutations (0.39; 95% CI, 0.13–0.66). Our findings are consistent with effects of both allelic and nonallelic familial factors on the clinical variability of NF2. Genet. Epidemiol. 23:245–259, 2002.

Key words: intrafamilial correlation; random effects model; right censoring; negative-binomial gamma mixture model; neurofibromatosis 2

INTRODUCTION

Variable expressivity is common in Mendelian diseases, especially those that are transmitted as autosomal-dominant traits. Variable expressivity may be manifested in many different ways, including variation in age at onset, types and numbers of clinical features that develop, overall disease severity, rate of progression, length of course, or final outcome. Many different genetic and nongenetic causes of variable expressivity may exist and act alone or in combination [Scriver and Waters, 1999; Dipple and McCabe, 2000].

Random effects models are used to estimate intraclass and intrafamilial associations by dividing phenotypic variance into components that are attributable to different sources of variation. Although methods based on sums of squares are widely used to estimate these variance components, this approach is not applicable when censoring is present. Moreover, since the standard random effects model is based on normality assumptions, it is not appropriate when the data are discrete. In this paper, we extended the standard random effects model to overcome these limitations. We demonstrate the use of these extended models by analyzing the familiality of selected clinical features of neurofibromatosis 2 (NF2).

NF2 is a highly penetrant Mendelian disease that is transmitted as an autosomal-dominant trait. The incidence of NF2 at birth has been estimated to be between 1 in 33,000 and 1 in 40,000 [Evans et al., 1992a]. Age at presentation is usually between 11–30 years, although younger cases and diagnoses in the fourth and fifth decades also occur [Evans et al., 1992a; Parry et al., 1994]. The hallmark of NF2 is bilateral vestibular schwannomas (VSs), but meningiomas, nonvestibular schwannomas, and presenile cataracts are also common. NF2 symptoms are usually related to ''tumor burden,'' i.e., the number, size, and location of tumors, and may include hearing loss, tinnitus, vertigo, seizures, facial weakness, and visual impairment [Evans et al., 1992c; Parry et al., 1994].

The responsible gene, *NF2*, has been identified and sequenced [Trofatter et al., 1993; Rouleau et al., 1993]. Pathogenic mutations have been found throughout the gene, and a different mutation occurs in almost every family. These mutations are of

various types, but most can be classified as nonsense, frameshift, splice-site, missense, or large deletions [MacCollin, 1999].

Clinical studies indicate that the phenotypic expression and natural history of NF2 tend to be similar within a family, and that more variability occurs between families [Evans et al., 1992a; Parry et al., 1994, 1996]. Previous studies demonstrated allele-phenotype correlations for certain *NF2* mutation classes. In general, constitutional truncating mutations (frameshift or nonsense) are associated with severe disease, missense mutations and large deletions with milder disease, and splice-site mutations with variable disease severity, although exceptions do occur [Kluwe et al., 1996, 1998; Parry et al., 1996; Ruttledge et al., 1996; Evans et al., 1998a].

Despite the general similarity in disease severity among affected relatives, substantial phenotypic differences may occur within families [Mautner et al., 1996; Baser et al., 1996b]. It is not known whether this variability occurs by chance or is caused by modifying genes at other loci [Bruder et al., 1999], coincident environmental exposures, or some combination of factors [Baser et al., 1996b].

We developed statistical methods to estimate the magnitude of intrafamilial correlations for continuous variables with censored observations and for count variables. We used these methods to test whether the phenotypic similarities found among relatives with NF2 can be explained entirely by the recognized *NF2* mutation class-phenotype correlation. We calculated intrafamilial correlation coefficients (τ) for three clinical features (age at first symptom, age at onset of hearing loss, and number of intracranial meningiomas) for a large series of NF2 patients and within subgroups of patients with truncating mutations, splice-site mutations, missense mutations, or large deletions of the *NF2* gene. We demonstrate significant intrafamilial correlations for each of these phenotypic features within the entire group of NF2 patients and in one or more subgroups of patients with a particular class of constitutional *NF2* mutations. Our findings suggest that familial factors beyond *NF2* mutation class are important in the pathogenesis of these features in some patients with NF2.

MATERIALS AND METHODS

Statistical Analysis

Random effects model for censored data

In a random effects model, the total variance for a variable can be separated into two components: variance between families (σ_B^2) and variance within a family (σ_W^2). Let k be the number of families in the study, n_i be the number of affected members in the ith family, and Y_{ij} be the value of the jth patient of the ith family. The statistical model is

$$Y_{ij} = \mu + A_i + \varepsilon_{ij},\ i = 1, \ldots k;\ j = 1, \ldots n_i,$$

where A_is are independent normal random variables with mean 0 and variance σ_B^2; ε_{ij}s are also independent random variables with mean 0 and variance σ_W^2. A_is and ε_{ij}s are mutually independent. In the above model, μ represents the overall mean of all the individuals; A_i is common to all the members from the same family, representing

the deviation of the mean of this particular family from the overall mean μ. The variance of A_i, σ_B^2, reflects the between-family variation, and the variance of ε_{ij}, σ_W^2, reflects the within-family variation. The total variance σ^2 is the sum of σ_B^2 and σ_W^2. When the feature is relatively homogenous within families, σ_W^2 will be small in comparison to the total variance. Therefore, the strength of intrafamilial resemblance can be measured by the ratio of the between-family variance to the total variance: $\tau=\sigma_B^2/(\sigma_B^2 + \sigma_W^2)$, i.e., the intrafamilial correlation.

A widely used procedure for estimating variance components is to equate sums of squares to their expected values; this approach is not applicable when the variable under consideration is subject to right censoring. Therefore, we used maximum likelihood estimation (MLE) to incorporate survival techniques into a random effects model. Each family in the study contributes one term to the likelihood function. For an individual who has developed the age-dependent feature, we calculate the instantaneous likelihood that the feature occurs at the observed onset age; for an individual who does not have the feature, we calculate the likelihood that the feature occurs beyond the patient's current age. For the ith family, let T_i be the subgroup of all the individuals with the feature and C_i the subgroup of all individuals without the feature. y_{ij} is the age at onset of the feature if it is present; otherwise, y_{ij} is the patient's age at last examination. The contribution of the family to the likelihood is

$$P_i = f_{T_i}(y_{ij}, j \in T_i)\,\Pr(Y_{ij'} > y_{ij'}, j' \in C_i | Y_{ij} = y_{ij}, j \in T_i),$$

where f_{T_i} is the joint density of $\{Y_{ij}, j \in T_i\}$, and the second term on the right-hand side is the conditional probability of $\{Y_{ij'} > y_{ij'}, j' \in C_i\}$ given $\{Y_{ij} = y_{ij}, j \in T_i\}$. P_i is parametrized as a function of μ, σ^2 and τ [Jobson, 1996]. The log-likelihood $\Sigma\log(P_i)$ can be maximized numerically with a quasi-Newton method (e.g., Nash, 1990) to obtain the maximum likelihood estimates of μ, σ^2 and τ together with an estimated covariance matrix.

We applied this method to data for two continuous variables available on NF2 patients: age at first symptom and age at onset of hearing loss. For age at first symptom, censoring is present when a patient is asymptomatic at the time of examination or death; for age at onset of hearing loss, censoring occurs when a patient does not have hearing loss at the time of examination or death.

Random effects model for discrete data

A random effects model based on a normal distribution is not realistic for a count variable with a high frequency of zeros, such as number of meningiomas in a patient with NF2. We considered using a Poisson distribution to model these data, but the mean and variance are equal in the Poisson distribution. In contrast, the within-family variation is greater than the mean in the NF2 meningioma data. We used a negative-binomial gamma mixture model, based on the assumption that the expected count may differ between families as well as within a single family. The similarity within families is represented by a factor with a gamma distribution. For any given family, the count in each member follows a negative-binomial distribution [Lawless, 1987] conditional on the familial factor.

Suppose in the ith family, Y_{ij} is the count in the jth member. We assume that the family factor Λ_i is an unobserved random variable having a gamma distribution with mean 1 and variance $1/\theta$. Conditionally on Λ_i, Y_{ij}s are independent and have a negative-binomial distribution with mean $\mu_{ij} = \mu_0\Lambda_i$, where μ_0 is the overall mean count across all the families. Given μ_{ij} and another parameter λ, the probability function of the negative-binomial distribution is fully specified as

$$\Pr(Y_{ij} = y) = \frac{\Gamma(\lambda + y)\mu_{ij}^{y}\lambda^{\lambda}}{\Gamma(\lambda)y!(\mu_{ij} + \lambda)^{\lambda+y}}.$$

Since the family factor Λ_i is a random variable, the count per patient varies from family to family. A large variance of Λ_i implies that the families are very different in their means. The correlation between two particular family members, τ, depends on θ, λ, and μ_0:

$$\tau = \frac{\mu_0^2\lambda}{\mu_0\theta\lambda + \mu_0^2(1 + \theta + \lambda)}.$$

The mean μ_{ij} can also be allowed to depend on covariates through a log link function. Let x_{ij} be a vector of covariates and β the vector of coefficients, then $\mu_{ij} = \Lambda_i \exp(x_{ij}\beta)$. The correlation between two particular family members is no longer a constant, but instead depends on their x-values. If the covariate values of two family members are x_{ij} and $x_{ij'}$, the correlation between them is:

$$\tau = \frac{\mu_j\mu_{j'}\lambda}{\sqrt{[\mu_j\theta\lambda + \mu_j^2(1 + \theta + \lambda)][\mu_{j'}\theta\lambda + \mu_{j'}^2(1 + \theta + \lambda)]}},$$

where $\mu_k = \exp(x_{ik}\beta)$, $k = j$ or j'.

Note that in the gamma negative-binomial model, the variance cannot be partitioned into additive components. The variance of the familial factor is $1/\theta$ and the conditional variance of the individual factor depends on the dispersion parameter $1/\lambda$. They are not additive because there is additional variation from the Poisson sampling that depends on the mean.

We used this negative-binomial gamma mixture model to assess familiality of meningioma count data in NF2 patients. The maximum likelihood estimates of θ, λ, and μ_0, together with an estimated covariance matrix, were obtained numerically using a quasi-Newton method [Nash, 1990], and the standard error of τ was derived by the delta method [Agresti, 1990]. It would be appropriate to include covariates such as age, but this information was unavailable for many patients in our data set. Therefore, no covariates were included in the analysis presented below.

Genotype-phenotype correlations

The constitutional *NF2* mutation was known in a subset of the families, and this permitted us to assess whether the *NF2* allele-phenotype correlation accounts for all of the intrafamilial correlation observed. Patients belonging to families with each of the following four kinds of *NF2* constitutional mutations were analyzed separately: 1) truncating mutations (frameshift or nonsense), 2) splice-site or splice effect mutations, 3) missense mutations, and 4) large deletions.

TABLE I. Number of NF2 Families and Patients Included for Each of the Clinical Features Examined

Mutation type and clincal feature	Number of families	Number of patients
All mutation types		
Age at first symptom	150	373
Age of onset of hearing loss	114	261
Number of intracranial meningiomas	122	259
Truncating (nonsense or frameshift)		
Age at first symptom	37	58
Age of onset of hearing loss	25	39
Number of intracranial meningiomas	30	44
Splice-site		
Age at first symptom	32	101
Age at onset of hearing loss	23	60
Number of intracranial meningiomas	27	79
Missense		
Age at first symptom	12	50
Age at onset of hearing loss	9	38
Number of intracranial meningiomas	10	23
Large deletions		
Age at first symptom	13	42
Age at onset of hearing loss	11	34
Number of intracranial meningiomas	12	36

Intrafamilial correlation coefficients were calculated within subsets of families who shared similar constitutional *NF2* mutation types. To demonstrate the *NF2* genotype-phenotype correlations, we also compared the means of each pair of mutation subgroups simultaneously. The Bonferroni method [Seber, 1977] was used to control the type I error in these multiple comparisons. The z-score was calculated for the difference between each pair of means, but only those with P-value $< \alpha/k$ were considered to be statistically significant, where α was chosen as 0.05, and k is the total number of pairs tested (6 in this instance).

Patients

Three hundred and ninety patients from 153 families were ascertained from both published and unpublished sources (Supplemental information can be found at http://www.interscience.wiley.com/jpages/0741-0395/suppmat/index.html). All patients included met the Manchester clinical diagnostic criteria for NF2 [Evans et al., 1992b], had an identified constitutional *NF2* mutation, or both. Probands were excluded from the table and from all statistical analyses to avoid ascertainment bias. All other affected individuals were included if clinical information was available for at least 1 of the 3 manifestations studied: age at first symptom, age at onset of hearing loss, or number of intracranial meningiomas. These variables were examined because they were the most reliably reported features across the various data sources used for the study. Meningiomas were identified by cranial CT or MRI scan. Only intracranial meningiomas were considered in this study. The total numbers of families and patients used to examine each clinical feature are given in Table I.

Age at first symptom of NF2 and age at onset of hearing loss are both subject to right censoring. Censoring can occur either because the manifestation was not present at the time of last evaluation, or because the manifestation was not present when the subject died. Death accounts for a small proportion of censored cases in this data set.

RESULTS

Among the 390 NF2 patients included in this study, 300 (76.9%) had bilateral VSs, 31 (7.9%) had a unilateral VS, 26 (6.7%) had no VS, and in 33 cases (8.5%) the VS status was unknown.

Age at First Symptom

Three hundred and seventy-three patients from 150 families were included in the study of age at first symptom. Seventy-two (19%) patients were asymptomatic at time of last examination or death, and were therefore treated as right-censored cases in this analysis. Among the symptomatic patients, age at first symptom ranged from 1–62 years.

To assess the assumption of normality for age at first symptom, we examined normal probability plots for all subjects together and for subjects in each mutation subclass. These plots did not show extreme skewness, except in the subclass of patients with large deletion mutations, where the distribution was skewed to the right. The random effects model was also fit in this subgroup, using log-transformed age at first symptom. The estimate of τ was about the same, so only the results of the model using untransformed values of age are reported here.

Table II shows the means, standard deviations, and intrafamilial correlations calculated for affected members of all families included in this study, as well as for members of families with each of four types of constitutional *NF2* mutations: truncating mutations, splice-site mutations, missense mutations, and large deletions. The value of τ within each subgroup of mutations except large deletions was similar in magnitude to that seen when all families were analyzed together. For all *NF2* mutations considered together, the intrafamilial correlation coefficient for age at first symptom was 0.35, and the lower 95% confidence limit was 0.23. The 95%

TABLE II. Mean, Standard Deviation, and Intrafamilial Correlation of Age at First Symptom in 373 NF2 Patients From 150 Families[a]

Mutation type	Censoring rate	Mean	Standard deviation	Intrafamilial correlation (τ)
All mutation types	19%	24.9 (23.1, 26.8)	13.1 (12.0, 14.2)	0.35 (0.23, 0.47)
Truncating (nonsense or frameshift)	15%	18.7 (15.6, 21.9)	9.5 (7.6, 11.5)	0.41 (0.06, 0.68)
Splice-site	19%	25.1 (21.6, 28.5)	12.1 (10.0, 14.2)	0.29 (0.03, 0.51)
Missense	20%	29.3 (24.1, 34.6)	11.9 (8.9, 14.9)	0.32 (0, 0.61)
Large deletions	14%	24.5 (20.0, 29.0)	11.3 (8.7, 14.0)	0.10 (0, 0.34)

[a]Approximate 95% confidence intervals of point estimates are given in parentheses.

confidence intervals for τ were always wider in the subgroups, as expected with smaller sample sizes. Nevertheless, in two of the subgroups (truncating mutations and splice-site mutations), the lower limit of the 95% confidence interval of τ excluded 0.

We conducted pairwise tests to assess differences between mean ages at first symptom in the subgroups, and tested nominal statistical significance using the Bonferroni method. The mean age at first symptom in the subgroup with truncating mutations was significantly different from the mean age at first symptom in the splice-site and missense subgroups, whereas the differences between all other pairs were not statistically significant. Patients with truncating mutations had an earlier mean age at first symptom (18.7 years) and less variation (standard deviation, 9.5 years). The pattern of age at first symptom is shown more clearly in the Kaplan-Meier estimates of the proportion of asymptomatic patients at various ages for each mutation type (Fig. 1).

Age at Onset of Hearing Loss

Of 261 NF2 patients from 114 families for whom hearing status was known, 192 individuals (74%) had lost their hearing at the time of examination. The age at onset of hearing loss among these patients ranged from 3–62 years. Sixty-nine (26%) of the 261 patients did not have hearing loss at the time of last examination or death, and were treated as right-censored in the analysis.

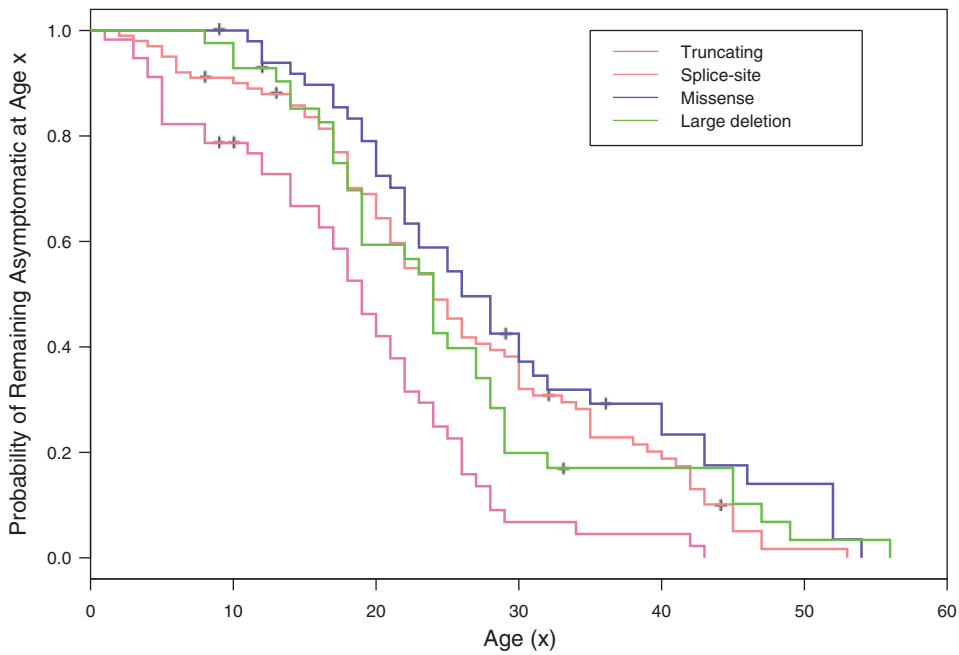

Fig. 1. Kaplan-Meier estimates of probability of remaining asymptomatic at a given age. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com]

TABLE III. Mean, Standard Deviation, and Intrafamilial Correlation of Age at Onset of Hearing Loss for 261 NF2 Patients From 114 Families[a]

Mutation type	Censoring rate	Mean	Standard deviation	Intrafamilial correlation (τ)
All mutation types	26%	29.6 (27.2, 32.1)	13.3 (11.8, 14.9)	0.51 (0.35, 0.64)
Truncating (nonsense or frameshift)	23%	22.2 (19.3, 25.1)	7.4 (5.5, 9.4)	0.41 (0, 0.76)
Splice-site	40%	31.6 (27.4, 35.9)	11.7 (8.9, 14.5)	0.29 (0, 0.62)
Missense	24%	36.9 (27.4, 46.4)	14.9 (8.1, 21.7)	0.67 (0.18, 0.89)
Large deletions	26%	28.9 (22.2, 35.6)	13.0 (9.2, 16.8)	0.19 (0, 0.55)

[a]Approximate 95% confidence intervals of the point estimates are given in parentheses.

To assess the assumption of normality for age at onset of hearing loss, we examined normal probability plots for all patients together and for each mutation subclass. The distribution for all cases together was not skewed, but right skewness was observed for all subclasses except truncating mutations. A logarithmic transformation provided a better fit for the subgroups that had a skewed distribution, but the estimates of τ remained almost the same as without the transformation. For this reason, we only report results for the analysis without transformation of age.

The means, standard deviations, and intrafamilial correlations for age at onset of hearing loss are reported in Table III, and the Kaplan-Meier estimates are plotted in Figure 2. A strong intrafamilial correlation was seen for age at hearing loss when all patients were considered together ($\tau = 0.51$; 95% CI, 0.35–0.64). Within the subgroups defined by constitutional *NF2* mutation type, those with missense mutations had a somewhat higher intrafamilial correlation than the other subgroups, and it was only in this subgroup that the 95% confidence interval of the correlation coefficient excluded zero.

Pairwise tests showed that the mean age at onset of hearing loss for patients with truncating mutations was significantly lower than that of patients with splice-site or missense mutations. The means of the other subgroups did not differ significantly from each other.

Number of Intracranial Meningiomas

Two hundred and fifty-nine NF2 patients from 122 families were used in the study of intracranial meningiomas. The distribution of number of meningiomas is summarized in Table IV by mutation type, and estimates of the model parameters and intrafamilial correlations are reported in Table V.

The mean number of intracranial meningiomas per patient was 1.01 (95% CI, 0.70–1.32). A significant intrafamilial correlation for number of meningiomas was observed for all NF2 patients combined ($\tau = 0.29$; 95% CI, 0.15–0.43).

NF2 patients with truncating mutations had the highest mean number of meningiomas, i.e., 1.92 (95% CI, 1.02–2.82), but this was associated with relatively high within-family variance. The magnitude of the intrafamilial correlation coefficient was small in this subgroup.

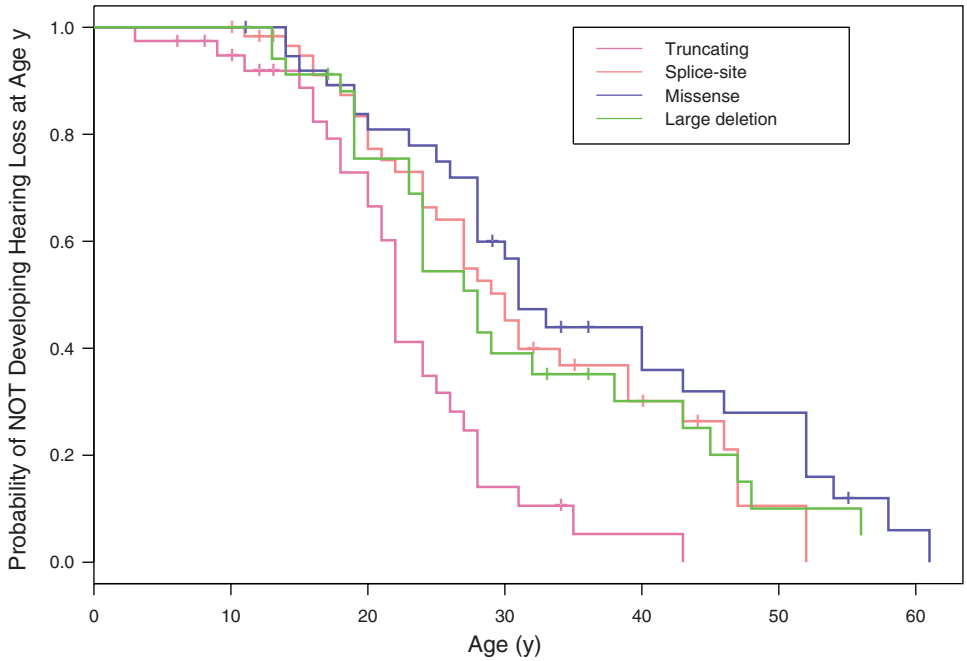

Fig. 2. Kaplan-Meier estimates of probability of *not* developing hearing loss by a given age. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com]

TABLE IV. Distribution of Number of Meningiomas in 259 NF2 Patients From 122 Families

	Frequency of number of meningiomas										
Mutation type	0	1	2	3	4	5	6	7	9	10	19
All mutation types	164	50	19	8	6	5	2	1	2	1	1
	63.3%	19.3%	7.3%	3.1%	2.3%	1.9%	0.8%	0.4%	0.8%	0.4%	0.4%
Truncating[a]	18	9	4	4	4	1	1	1	1	1	0
	40.9%	20.5%	9.1%	9.1%	9.1%	2.3%	2.3%	2.3%	2.3%	2.3%	
Splice-site	50	19	6	1	1	1	0	0	1	0	0
	63.3%	24.1%	7.6%	1.3%	1.3%	1.3%			1.3%		
Missense	16	6	0	1	0	0	0	0	0	0	0
	70.0%	26.1%		4.3%							
Large deletions	25	4	6	0	0	0	0	0	0	0	1
	69.4%	11.1%	16.7%								2.8%

[a]Nonsense and frameshift.

The mean number of intracranial meningiomas among NF2 patients with splice-site mutations was 0.72 (95% CI, 0.29–1.15). In contrast to the situation with truncating mutations, the within-family variation was small, and the between-family variation was large for NF2 patients with splice-site mutations. The point estimate of

TABLE V. Parameter Estimates and Intrafamilial Correlation Coefficients for Number of Meningiomas[a]

Mutation type	$1/\theta$[b]	$1/\lambda$[c]	Mean (μ_0)	Intrafamilial correlation (τ)
All mutation types	1.24 (0.53, 1.95)	0.93 (0.32, 1.54)	1.01 (0.70, 1.32)	0.29 (0.15, 0.43)
Truncating (nonsense and frameshift)	0.29 (0.00, 0.58)	1.19 (0.17, 2.21)	1.92 (1.02, 2.82)	0.12 (0.00, 0.25)
Splice-site	1.43 (0.14, 2.72)	0.34 (0, 0.89)	0.72 (0.29, 1.15)	0.39 (0.13, 0.66)
Missense	0.28 (0, 0.69)	0.47 (0, 2.45)	0.42 (0.09, 0.75)	0.08 (0, 0.23)
Large deletions	1.25 (0, 3.94)	2.37 (0, 5.96)	0.96 (0, 2.02)	0.16 (0, 0.45)

[a]Approximate 95% confidence intervals for the point estimates are given in parentheses.
[b]$1/\theta$, variance between families.
[c]$1/\lambda$, negative binomial dispersion parameter.

the intrafamilial correlation coefficient was higher in this subgroup than in any of the other mutation subgroups, and the 95% confidence interval excluded zero.

The mean number of intracranial meningiomas among NF2 patients with missense mutations was 0.42 (95% CI, 0.09–0.75), the lowest among the four mutation subgroups because 16 of the 23 patients in this subgroup had no meningiomas. The variation both between families and within a family was similar in magnitude to the mean, and the intrafamilial correlation coefficient was small.

The mean number of intracrainal meningiomas among NF2 patients with large deletions was 0.96 (95% CI, 0–2.02). The within- and between-family variances were both large, mainly because of one patient (patient 201 in family 1648) who developed 19 meningiomas by age 18 [Bruder et al., 2001]. More than 2/3 of individuals with this mutation type had no meningiomas, and all of the others had either one or two meningiomas, including patient 201's two affected relatives. When this family was excluded from the analysis, the mean number of meningiomas among the remaining patients with large deletions was 0.39, both within- and between-family variation were much smaller, and the intrafamilial correlation was even lower (0.06).

DISCUSSION

Statistical Methods

The statistical methods used here should be of use in intrafamilial correlation studies of other genetic diseases. Random effects models are commonly used to analyze intraclass and intrafamilial correlations in continuous traits, and we extended this method to include right-censored data. The maximum likelihood method we describe can also accommodate two other types of censoring frequently associated with age-related traits: left censoring (e.g., the event occurred before time of examination) and interval censoring (e.g., the event occurred between two examinations). A mixed-effects model can be used to adjust the correlations calculated by this method for covariates [Searle et al., 1992].

The negative-binomial gamma mixture model we developed for count traits is also likely to be useful for other genetic diseases. A Poisson mixture model is sometimes used with count data [Foulley et al., 1987], but the Poisson distribution

is constrained because the variance is equal to the mean. A mixture model based on the negative-binomial distribution allows more flexibility, and is therefore more appropriate for count variables with overdispersion relative to the Poisson distribution [Tempelman and Gianola, 1996].

Intrafamilial Correlations in NF2

Phenotypic variability is observed in individuals with NF2, both within and between families. We employed a random effects model incorporating survival techniques to estimate intrafamilial correlations in two continuous variables that are right-censored: age at first symptom, and age at onset of hearing loss. We used a negative-binomial gamma mixture model to estimate intrafamilial correlations for a discrete variable, i.e., number of intracranial meningiomas. Our results demonstrate that relatives with NF2 are more similar to each other than to unrelated affected individuals with respect to each of these clinical features. These observations are consistent with anecdotal clinical experience [Evans et al., 2000]. Parry et al. [1996] adjusted for intrafamilial correlation in their genotype-phenotype analysis, but the intrafamilial correlation of NF2 phenotypes has not previously been tested statistically.

Intrafamilial correlations, such as those observed in this study, may have a variety of causes. Effects of the mutant allele, of other shared genes, of shared environmental factors, or of a combination of genetic and environmental factors may produce such correlations. Distinguishing between these possibilities requires analysis of phenotypic correlations among affected family members of various classes, such as monozygotic twins, sibs, parent-child pairs, and more distant relatives.

Since all affected individuals in the same family can be presumed to carry the same constitutional alteration of the *NF2* locus, the nature of the *NF2* mutation itself might account for the familiality we observed. This possibility is supported by the associations observed in cross-sectional studies between allele class and disease severity in NF2 [Kluwe et al., 1996, 1998; Parry et al., 1996; Ruttledge et al., 1996; Evans et al., 1998a]. Our study includes data on patients who are also included in these earlier studies, and as expected, we found similar effects.

We also observed intrafamilial correlations of similar or greater magnitude for each of the features studied in subgroups of patients who all had the same type of constitutional *NF2* mutation. While it is possible that specific allelic differences within each mutation class account for these intrafamilial correlations, our findings could also reflect the effects of modifying genes. Recent reports of putative modifying loci for *NF2* are consistent with this interpretation [Bruder et al., 1999; Goutebroze et al., 2000]. Several genes other than *NF2* have been implicated in meningioma development, including loci on chromosomes 1, 3, 6, 7, and 22 [Sanson et al., 1993; Sulman et al., 1998; Comtesse et al., 1999], but the contribution of these loci to the interfamilial variability observed in NF2 pedigrees is unknown.

Our studies are subject to several limitations. We used data from a variety of sources, and differences in referral patterns, diagnostic acumen, and criteria for diagnosis probably exist among the centers. Age at first symptom and age at onset of hearing loss were taken from published data (including updated data provided by the

authors) and unpublished data. The definitions of these ages may vary from source to source. In many cases, age at first symptom and age at onset of hearing loss were assigned retrospectively and thus may be subject to recall errors. All these factors could affect the accuracy of our results.

Although this study was based on the largest collection of clinical data available on NF2 patients, consideration of separate mutation types was limited by small sample sizes. Consequently, our estimates of τ for the subgroups are associated with wide confidence intervals. Some of the correlations that did not appear to be significant in this study might be important, but require larger samples for demonstration. A likelihood ratio test could be performed to assess whether intrafamilial correlations vary significantly with mutation type. This would be useful in a study that included more patients, but a likelihood ratio test would not show significant differences because of the small sample size of each subgroup in the present study.

The penetrance of NF2 and the prevalence of individual tumor types generally increase with age [Mautner et al., 1993; MacCollin and Mautner, 1998]. Time from onset of symptoms may also influence the number of meningiomas in an NF2 patient, so it would be appropriate to model this time variable as an additional source of variation that is independent of the familial factor. Unfortunately, we did not know the age at which meningioma status was determined for many of the patients in this study, so we could not include age as a covariate in our analysis.

Statistical techniques provide powerful means of studying genetic and nongenetic aspects of diseases such as NF2. Methods are needed to estimate intrafamilial correlations for other kinds of non-normally distributed traits, such as ordered categorical data (e.g., severity of disease) and continuous data that are not normally distributed (e.g., disease progression rate). Each of these data types requires a different statistical model to capture specific distributional features. Models that allow a wide range of dependent structures, so that various genetic and environmental components of phenotypic variation can be assessed at the same time, are especially desirable.

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